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Figure 1

Schematic representation of human tenascin-C and of the strategy followed for the generation of BC2-like antibodies

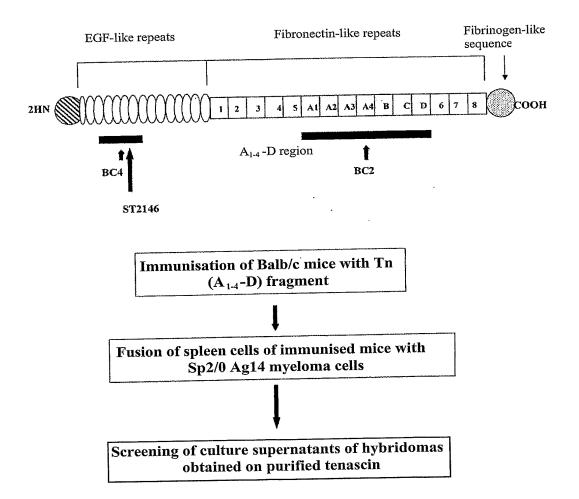
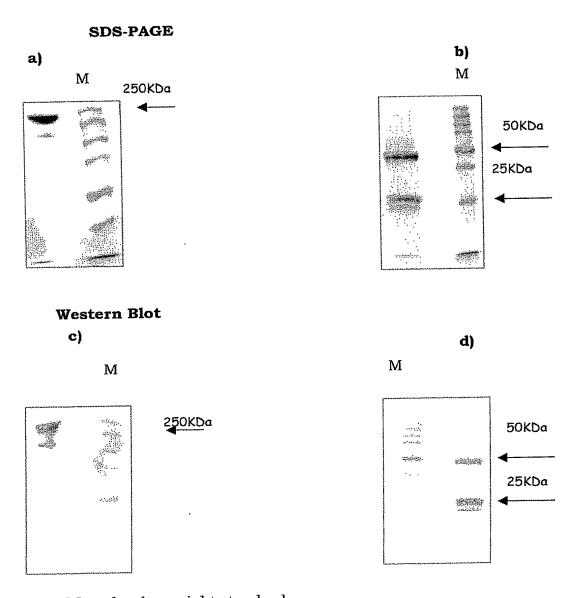


Figure 2

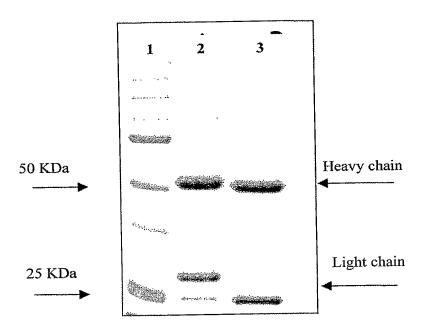
SDS-PAGE and Western Blot analysis of ST2485 antibody, in reducing (b, d) and non-reducing (a, c) conditions.



M: molecular weight standards

Figure 3

 ${
m ST2485}$ antibody digestion with Flavobacterium Peptide-N-glycosidase enzyme (PNGase F).

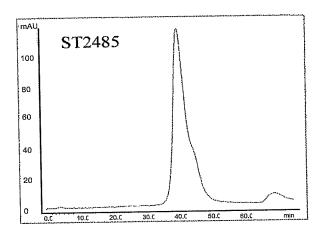


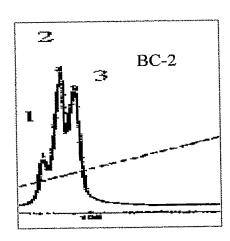
- 1: Molecular weight marker
- 2: Non-digested ST2485
- 3: PNGaseF-digested ST2485

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Figure 4

BC-2 and ST2485 antitenascin antibodies hydroxyapatite chromatography.

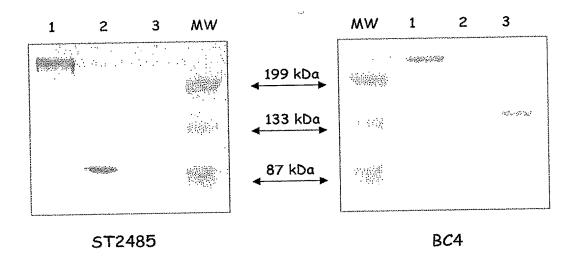




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Figure 5

ST2485 antitenascin antibody Western Blot Analysis



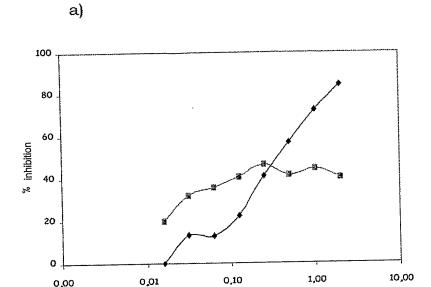
- 1: Tenascin-C
- 2: Tn A₍₁₋₄₎-D Fragment
- 3: Tenascin EGF-like region recombinant fragment, containing the epitope recognized by BC-4 antibody.

MW: molecular weight standards

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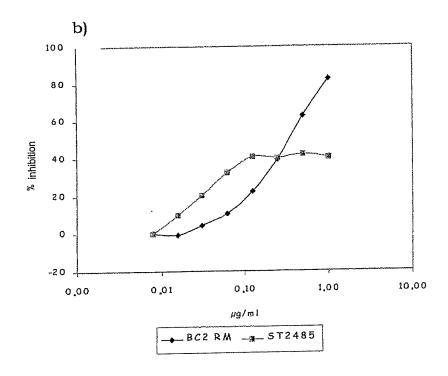
Figure 6

Competitive ELISA test between ST2485 and BC-2 for antigen binding.



0,01

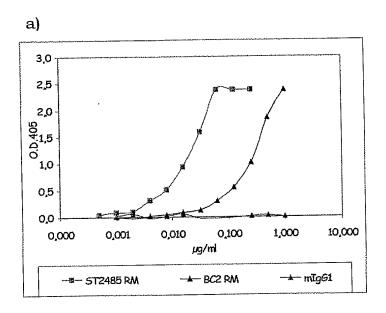
00,00

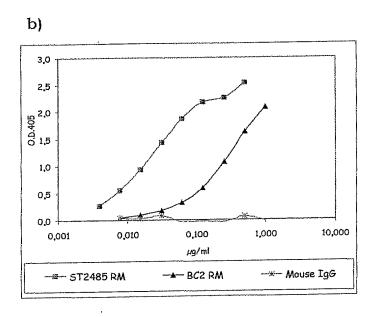


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Figure 7

Immunoreactivity of ST2485 antibody in comparison with BC-2, on tenascin C (a) and on Tn $A_{(1-4)}$ -D fragment (b).

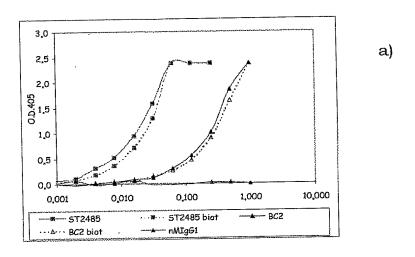


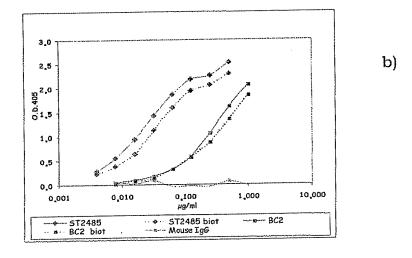


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Figure 8

Immunoreactivity of ST2485 and BC-2 biotinylated and non-biotinylated antibodies, on tenascin-C (a) and on Tn $A_{(1-4)}$ -D fragment (b).

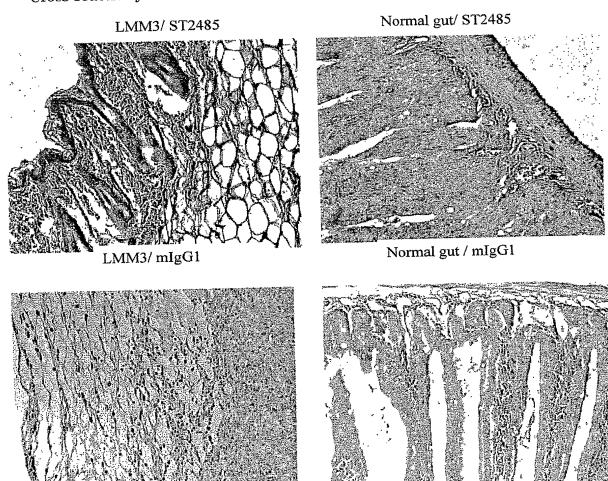




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Figure 9

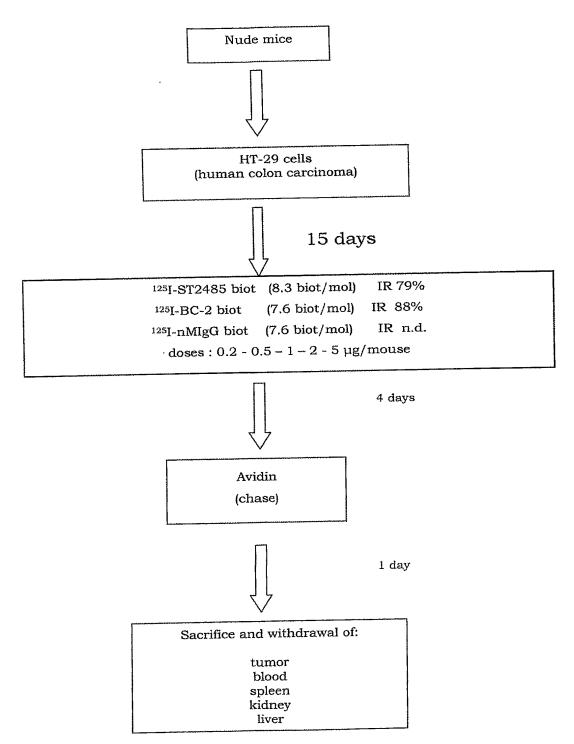
Cross-reactivity of ST2485 antibody with murine tenascin.



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Figure 10

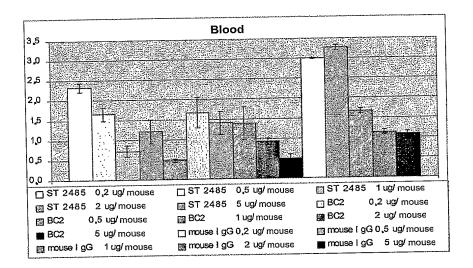
Biotinylated ST2485 and BC-2 antibodies biodistribution study protocol in human tenascin-expressing tumor-implanted nude mice.

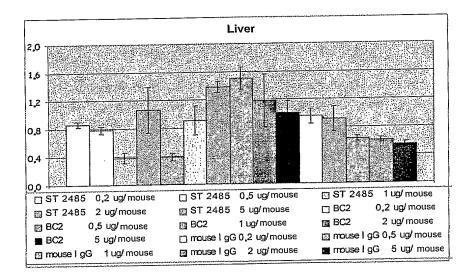


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Figure 11

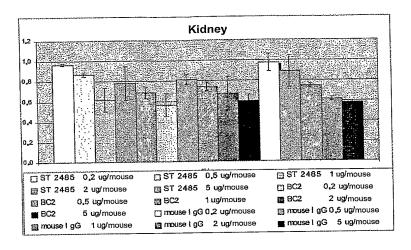
Biotinylated ST2485 and BC-2 biodistribution in human tenascinexpressing tumor-transplanted nude mice. The antibody amount is expressed as percent of the injected dose per tissue gram (% I.D./gr).

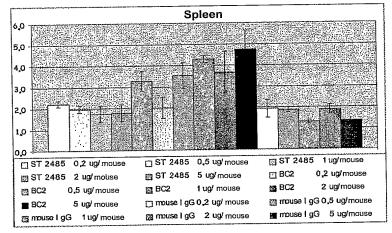


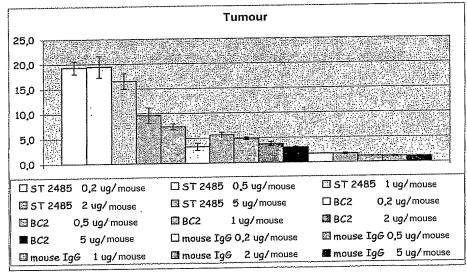


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Figure 11a



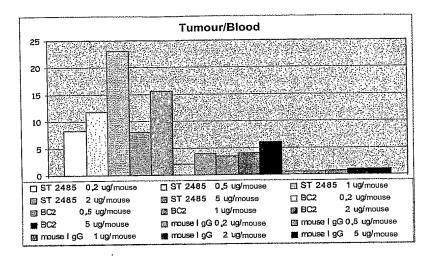


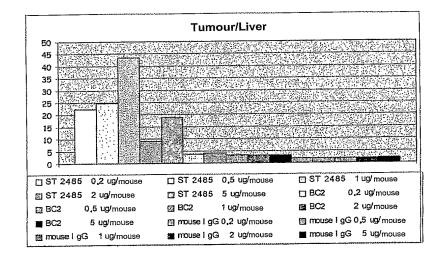


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Figure 12

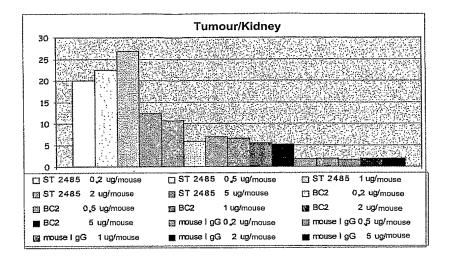
Biotinylated ST2485 and BC-2 biodistribution in nude mice: tumor/non tumor ratio.

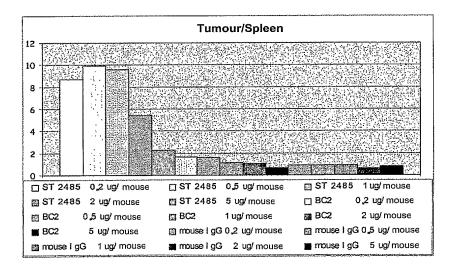




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Figure 12a



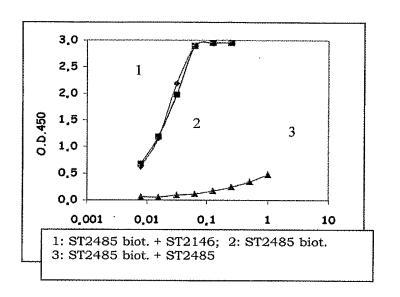


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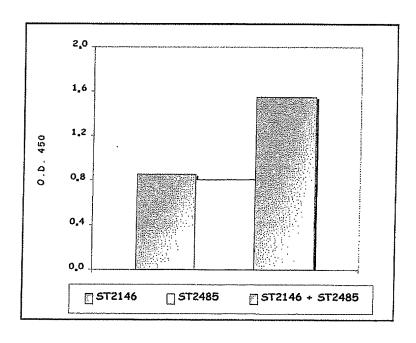
Figure 13

Interference (a) and additivity (b) ST2485 and ST2146 antitenascin antibodies in vitro evaluation by ELISA test.

a) Interference



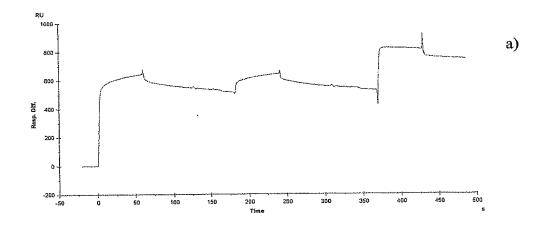
b) Additivity

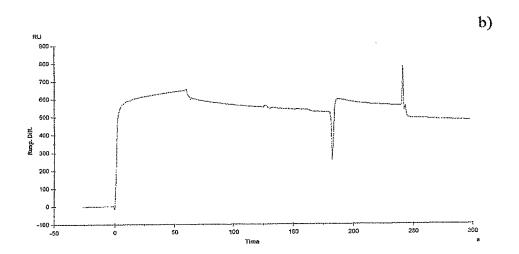


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Figure 14

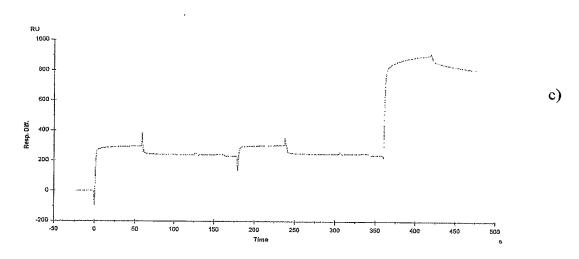
Antibodies ST2485 and ST2146 tenascin binding $in\ vitro$ additivity by BIACore

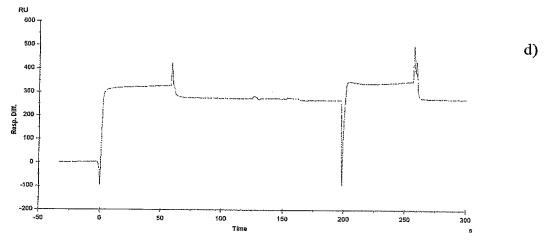




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Figure 14a





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Figure 15

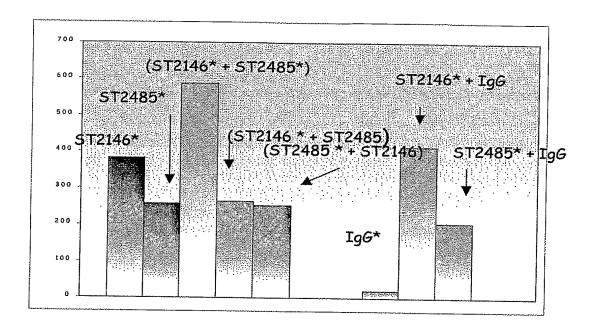
Schematic representation of in vivo additivity study in animal model

Tenascin-expressing human colon carcinoma (HT29)-transplanted nude mice Single or admixed radiolabelled biotinylated antibodies i.v. administration, 2ug/mouse dose each: 125I-ST2485 125I-ST2146 125I-ST2485 + 125I-ST2146 125I-ST2485+ ST2146 125I-ST2146+ ST2485 125I-ST2485+ nMIgG 125I-ST2146+ nMIgG 125I-nMIgG \Box 6 days avidin i.v. administration at a dose 100x with respect to antibody (chase) П 1 day Sacrifice and withdrawal of: tumor, blood, spleen, kidney, liver

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Figure 16

ST2485 e ST2146 antibodies additivity in animal model; tumor seat localization.



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Figure 17

SEQID 1 sequence of ST2485 kappa light chain variable region (VL).

Signal peptide

ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCTTCAGTCATAATGTCCAGAGGACAAA Met Asp Phe Glin Val Glin Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser Val Ile Met Ser Arg Gly Glin

TTGTTCTCCCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGGGGAGAAGGTCACAATGACTTGC lle Val Leu Ser Gln Ser Pro Ala lle Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys

N-glycosylation

CDR1

AGGGCAACTCAAGTGTACGTTTCATGCACTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACC

Arg Ala Asm Ser Ser Val Arg Phe Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys

CTGGATTATECAASCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGG
Pro Trp Ile Tyr Ala Tur Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly

GACCTCTTATTCTGTCACAATCAGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGC Ser Gly Thr Ser Tyr Ser Val Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln

AUTGGAGTAGTAATTCACCCAGGACGTTCGGTGGAGGCACCAAGGTGGAAATCAGACGGGCT Gln Trp Ser Ser Asn Ser Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Arg Arg Ala

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Figure 18

SEQID 2 sequence of ST2485 gamma heavy chain variable region (VH)

Signal peptide

ATGGGATGGAGCTGGATCTTTCTCTCCTCCTGTCAGGAACTGCAGGTGTCCACTCTGAGGTCCAGCTG
Met Glv Trp Ser Trp Ile Phe Leu Phe Leu Leu Sor Glv Thr Ala Glv Val His Ser Glu Val Gln Leu

CAACAGTCTGGACCTGAGCTGGAAGCCTTGAAGGATTTCCTGCAAGGCTTCTGG Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Met Lys Ile Ser Cys Lys Ala Ser

CDR1
TTACTCATTCAC TOTAL ACACCATGAACTGGGTGAAGCAGAGCCATGGAAAGAACCTTGAATGGA
Gly Tyr Ser Phe Thr Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp

TTGGACTT THE ASIA PRO His Asia Gly Gly Thr Thr Tyr Asia Glin Lys Phe Lys Gly Lys Ala Thr

TTAACTGTAGACAAGTCATCCAACACACACCCTACATGGAGCTCCTCAGTCTGACATCTGAGGACTC Leu Thr Val Asp Lys Scr Ser Asn ThrAla Tyr Met Glu Leu Ser Leu Thr Ser Glu Asp

TGCAGTCTATTACTGTACAAGAGGGGGGGTTACTACTGGTTCTTCGATGTCTGGGGCGCAGGGA Ser Ala Val Tyr Tyr Cys Thr Arg Pro Gly Gly Tyr Tyr Trp Phe Phe Asp Val Trp Gly Ala Gly

CCACGGTCACCGTCTCCTCA Thr Thr Val Thr Val Ser Ser